

## PATENT

CERTIFICATE UNDER 37 C.F.R. §1.10

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Timothy K. Ball

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: Authorized Officer: Anne R. Kubilek

In response to the Written Opinion, the Applicant herewith submits substitute sheets corresponding to the amended claims in the above captioned application as filed. The substitute claims correspond to the previously presented claims except that the limitation to "specific hybridization conditions" has been deleted from claim 1 and claims dependent on claim 1, and nucleotide sequences that do not encoded at least an insecticidal fragment of a B. thuringiensis insecticidal toxin are also deleted. The amended claims are believed not to extend beyond the scope of the claims as originally presented. The substitute claims are believed to be directly and unambiguously supported by the specification as filed.

It is believed that the claims as amended now exhibit novelty in view of Berry et al. and Whang et al. and do not now lack inventive step in view of Berry et al. in view of PGS Patent Application No. EP 0 451 878 A1.

## REMARKS

### UNITY OF INVENTION

The Examiner has indicated that the claims lack unity of invention under PCT Rule 13. The Applicant traverses this assertion.

The claims, in general, are directed to isolated polynucleotide sequences that encode *B. thuringiensis* insecticidal toxins or insecticidal fragments thereof, and to nucleotide sequences that hybridize to such nucleotide sequences, the sequences being selected from the group consisting of SEQ ID NO's 2, 3, 5, 7, and 9. These nucleotide sequences, and insecticidal proteins encoded therefrom, exhibit unity of invention because each exhibit a high degree of identity when compared to each other, both at the amino acid sequence level and at the nucleotide sequence level. These genes each are obtained from *Bacillus thuringiensis* species of bacteria, and each encode a protein that contains an amino terminal signal sequence that targets the protein for secretion into the extracellular space of the bacterium. While other proteins are known to be secreted from *Bacillus* species, these proteins despite being highly related in size and sequence information, each exhibit insecticidal activity directed to coleopteran insect species. These sequences can be used alone or in combination in compositions of matter to achieve control of coleopteran insect infestations. In particular, sequences encoding these proteins can be inserted into plant species alone or in combinations the encoded proteins can be expressed in the plants to achieve protection of such transformed plants from coleopteran insect infestations. Therefore, it is believed that these sequences, both nucleotide and their encoded amino acid sequences, and the subject matter claiming these sequences, are so linked as to form a single general inventive concept under PCT Rule 13.1 and therefore DO NOT LACK UNITY OF INVENTION.

The Examiner has also drawn an incorrect reference to a protein disclosed in WO 01/87940, i.e., tIC851, which is also an insecticidal protein of *B. thuringiensis* exhibiting coleopteran insecticidal activity. However, tIC851 is a protein that is not secreted from *Bacillus*, and forms a unique parasporal crystalline structure. tIC851, when aligned vs any of the proteins disclosed in the instant specification, does not exhibit any relationship that a skilled artisan would recognize as one that would be construed to result in a conclusion that the proteins are in any way related within any phylogenetic tree. Therefore, it is believed that despite the Examiner's assertion that tIC851 is related to any of the proteins of the present invention, or her assertion that the *B. thuringiensis* gene encoding tIC851 would hybridize to any of the nucleotide sequences of the present invention is just not correct.

### NOVELTY

The Examiner has asserted that the claims 1-4, 12, 14, and 22-23 lack novelty because they are anticipated by Hwang et al. or Berry et al. For both references the Examiner has indicated that SEQ ID NO:5 lacks novelty in view of a sequence described in these references. The Applicant traverses this assertion as well.

SEQ ID NO:5 encodes an insecticidal protein referred to in the specification as TIC1201. The nucleotide sequence to which the Examiner has aligned SEQ ID NO:5 appears to correspond to a sequence that does not encode any protein. The Examiner has aligned SEQ ID NO:5 to nucleotide position 76521-76101 of the megadalton plasmid derived from an *israeliensis* subspecies of Bt. None of the sequences that the Examiner has indicated in her Result 1, that appear to exhibit any semblance of an open reading frame, appear to lie within the segment that the Examiner has aligned vs SEQ ID NO:5. The segment that the Examiner has aligned from the megadalton plasmid is not indicated to exhibit any potential for

encoding an insecticidal protein toxic to a coleopteran insect species. Furthermore, the sequence to which the Examiner has aligned SEQ ID NO:5 is not identical to SEQ ID NO:5 and therefore cannot anticipate SEQ ID NO:5. To destroy novelty, the reference must teach each and every limitation of the subject matter claimed. None of the proteins disclosed by Berry et al, nor the nucleotide sequence disclosed by Berry et al destroys the novelty of SEQ ID NO:5.

The novelty of SEQ ID NO:5 is also not destroyed by Hwang et al. The segment of Hwang et al to which the Examiner has aligned SEQ ID NO:5 is substantially within the NON-CODING segment 3' of the coding segment disclosed by Hwang et al. Therefore, the Hwang et al. segment that the Examiner asserts is anticipatory is in fact not a segment that encodes any insecticidal protein, and therefore, cannot anticipate SEQ ID NO:5 or any other sequence disclosed by the instant application.

#### INVENTIVE STEP

The Examiner has indicated that claims 1-4, 11-12, 14, and 22-23 lack inventive step over Berry et al in view of Plant Genetic Systems EP 0 451 878.

For the same reasons as above, Berry et al. does not exhibit a nucleotide sequence encoding an insecticidal protein toxic to coleopteran species of insects that hybridizes to SEQ ID NO:5. The '878 reference teaches to contact coleopteran pests with crystalline insecticidal toxins of *B. thuringiensis* species. Neither reference alone or in combination therefore renders the subject matter of the claims of the instant application obvious, and therefore the claims do not lack inventive step.

It is respectfully requested that the International Bureau enter the substitute sheets of claims.

Respectfully submitted,



Timothy K. Ball, Ph.D., Esq.

Reg. No. 42,287

For Monsanto Company

800 North Lindbergh Boulevard

Mail Zone E2NA

St. Louis, Missouri 63167

(314) 694-5811

(314) 694-5311 (fax)

**What is Claimed is:**

1. An isolated polynucleotide which encodes a *Bacillus thuringiensis* insecticidal toxin or insecticidal fragment thereof, wherein said polynucleotide hybridizes under ~~conditions selected from the group consisting of~~ stringent hybridization conditions ~~and specific hybridization conditions~~ with one or more of the nucleotide sequences selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3 (tic901), SEQ ID NO:5(tic1201), SEQ ID NO:7 (tic407), <sup>AND</sup> SEQ ID NO:9 (tic417), ~~SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:32~~ or with the complement thereof.
2. The isolated polynucleotide of claim 1 wherein said toxin is active against a coleopteran insect pest.
3. The isolated polynucleotide according to claim 2 wherein said coleopteran insect pest is selected from the group consisting of a corn rootworm and a Colorado potato beetle.
4. The polynucleotide according to claim 3 wherein said corn rootworm is selected from the group consisting of a western corn rootworm, a southern corn rootworm, or a northern corn rootworm.
5. The polynucleotide according to claim 1 wherein said nucleotide sequence is SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, <sup>and</sup> SEQ ID NO:9 ~~and SEQ ID NO:32~~
6. A polynucleotide comprising a nucleotide sequence which encodes an approximately 34 to about 39 kDa toxin active against a coleopteran pest, wherein said nucleotide sequence has been optimized for expression in plants, and wherein said toxin comprises the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, ~~SEQ ID NO:31, and SEQ ID NO:33~~ <sup>and</sup>
7. A host cell transformed to contain a polynucleotide encoding an insecticidal protein or insecticidal fragment thereof wherein said polynucleotide comprises a nucleotide sequence as set forth in a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, <sup>and</sup> SEQ ID NO:9 ~~SEQ ID NO:13, SEQ ID NO:30, and SEQ ID NO:32~~
8. The host cell of claim 7 wherein said host cell is a plant cell.

9. A recombinant bacterium comprising an isolated polynucleotide that encodes an insecticidal protein wherein said protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, ~~SEQ ID NO:31, and SEQ ID NO:33~~ <sup>and</sup> ~~SEQ ID NO:31, and SEQ ID NO:33~~

10. A method for controlling a coleopteran insect pest comprising contacting said pest with a pesticidal amount of an approximately 34 to about 39 kDa *Bacillus thuringiensis* toxin protein or insecticidal fragment thereof, wherein said toxin protein is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, ~~SEQ ID NO:31, and SEQ ID NO:33~~ <sup>and</sup> ~~SEQ ID NO:31, and SEQ ID NO:33~~

11. A method for controlling a coleopteran insect pest comprising contacting said pest with a pesticidal amount of an approximately 34 to about 39 kDa *Bacillus thuringiensis* toxin protein or insecticidal fragment thereof, wherein said toxin protein is encoded by a nucleotide sequence that is or that hybridizes under stringent conditions to a nucleotide sequence comprising at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, ~~SEQ ID NO:30, and SEQ ID NO:32~~ <sup>and</sup> ~~SEQ ID NO:30, and SEQ ID NO:32~~

12. An isolated insecticidal protein derived from *Bacillus thuringiensis* that is from about 34 kDa to about 39 kDa and is encoded by a nucleotide sequence that that is or that hybridizes under stringent conditions to a nucleotide sequence comprising at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, ~~SEQ ID NO:30, and SEQ ID NO:32~~ <sup>and</sup> ~~SEQ ID NO:30, and SEQ ID NO:32~~

13. An isolated insecticidal protein derived from *Bacillus thuringiensis* that is from about 34 kDa to about 39 kDa and is encoded by a nucleotide sequence that is or hybridizes under stringent conditions to at least 18 consecutive nucleotides selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, and SEQ ID NO:32, and wherein said *Bacillus thuringiensis* is selected from the group consisting of EG2158, EG6489, EG6561, EG3618, EG6555, EG6618, 86833, and EG4653.